

### REMARKS

Claims 1 through 14 and 16 through 49 were pending in the case at the time of the Office Action, with claims 18 through 21 having been previously withdrawn from consideration.

Claims 1-14, 16, 17 and 22-49 were rejected. In this response, claims 1, 3-5, 9-14, 16, 17, 22-27, 30-36, 39-46 and 48 have been amended to more clearly recite the invention. No new matter has been added by way of these amendments. Claims 7, 8, 15, 18-21, 28, 29, 37 and 38 have been cancelled herein without prejudice. Thus, claims 1-6, 9-14, 16, 17, 22-27, 30-36, 39-46, 48 and 49 are currently under consideration.

### Claim Objections

**Claim 1, 16, 17, 22 and 46 were objected to because of the following informalities:**

**Claims 1, 16, 17, 22 and 46 are missing article “A” or “The”.**

The claims have been amended herein to correct for the missing articles. The objection being therefore moot, withdrawal of the objection is respectfully requested.

**Claim 1 recites the phrase “evolved\_micro-organisms”. The underscore should be removed.**

The claim has been amended herein to correct for the typographical error. The objection being therefore moot, withdrawal is respectfully requested.

### Claim Rejections under 35 U.S.C. § 112

**Claims 1, 10, 12 and 16 and Claims 2-14, 17 and 32-49 depending therefrom were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1, 10, 12 and 16 recite the phrases “Evolved microorganisms”, “cause evolution”, “evolved genes”, and “Evolved strain”.**

This rejection is overcome, at least, for the following reasons.

The Office states that “evolution” is the change in genetic composition of an organism from one generation to the next and that it is therefore not clear how the “initial strain” comprising deletion of *tpiA* gene and a gene involved in conversion of methylglyoxal into lactate causes “evolution” or “evolved genes” in the same “initial strain”.

Applicants respectfully note that the Examiner is correct in identifying that “evolution” is a change that occurs genetically over one or more generations. Further, Applicants note that this

misunderstanding goes to the crux of the Office's rejection of the present invention under § 102 over Cameron. Specifically the evolution of the strain takes several generations of micro-organism growth. In this context, it should be appreciated that the life span of a microorganism is quite finite compared with those of multi-cellular organisms. For example, the doubling time of E. Coli grown in the laboratory is from 20 to 30 minutes. See for example, "Biology" – E. Coli (<http://en.allexperts.com/q/Biology-664/E-Coli-2.htm>). For yeast, the doubling time is approximately 1.5-2 hours. See, for example, "Yeast as a Model Eukaryote" (<http://drnelson.utmem.edu/yeastlect1.html>). Further the Office's attention is directed to Example 2 of the specification (paragraph [0203] of the published application US 2007/0072279) and Fig. 2. As discussed in paragraph [0203] the culture time allowed for the modified (e.g., the genetically altered "initial" strain) was several weeks. This time period is illustrated in Figs. 2A and 2B. Therefore, assuming a conservative estimate of 30 minutes per generational time for E. Coli, the time point at 200 hours represents 400 generations of E. Coli, the 800 hour time point 1600 generations etc. Similarly, the same time points for yeast culture would represent 100 generations, 400 generations etc. Therefore, the evolutionary process recited in the claims is, as noted by the Examiner taking place over many generation of the claimed microorganisms.

In addition, as explained in the specification at paragraphs [0042]-[0051] and recited in the claims this process begins initially with a wild type organism that is genetically modified to yield an initial microorganism. The initial microorganism is then grown on a defined medium to cause it to evolve (e.g., over a number of generations) as discussed above and as illustrated in Figs. 2A and 2B. The initial, modified microorganisms that have evolved are then selected to provide a strain of evolved cells capable of producing the desired end-product from the desired substrate. Thus, the process of "evolution", according to the invention, is defined by:

An initial strain: strain that has been genetically modified with a deletion of the *tpiA* gene and of at least one gene involved in the conversion of methyl glyoxal into lactate;

Successive steps: growth I a selective medium with a simple carbon source, under selective pressure, and selection of a strain having an increased 1,2-propanediol production;

An evolved strain: selected for having increased 1,2 propanediol production;

Thus, the product of the method is easily identifiable by its ability to produce 1,2 propanediol in greater yield, e.g., the activity of the enzyme is improved, compared to the initial strain.

**Claims 1 and 12 and Claims 2-13, 14, 17 and 32-49 depending therefrom were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1 and 12 recite the phrase “improved” activity.**

This rejection is overcome, at least, for the following reasons.

As discussed above, the identification of the evolved strain is easily accomplished because its production of 1,2-propanediol is greater, after evolution than is the production of 1,2-propanediol compared to the initial strain. This improved capacity is illustrated, for example in Fig. 2B where the concentration of products in the chemostat is illustrated over a period of 1400 hours beginning with the culture of the initial strain in the chemostat (see, paragraph [0075]). Because one of the features of chemostatic growth is that the volume is kept constant. Therefore, the bioreactor is continuously provided with fresh medium while culture medium is continuously removed. As illustrated in Fig. 2B, during the evolution phase of culture, the production of 1,2-propanediol continuously increases. Thus, as illustrated by Fig. 2B, the evolved strain has considerably improved production of 1,2-propanediol, that is, 1,2-propanediol synthase activity is much improved. In addition, the Applicants provide herewith data on three separate chemostat cultures of microorganisms prepared according to the invention. The data illustrates the improved activity of the 1,2-propanediol synthase in the evolved microorganism (units are gram of product/grams of biomass/time unit).

**Table 1S**

<b>ChemostatFcPG-02</b>	<b>Initial Strain</b>	<b>Evolved Strain</b>
Glucose consumption	0.13	0.18
1,2-propanediol production	0.02	0.06
1,2-propanediol and hydroxyacetone production	0.04	0.06

**Table 2S**

<b>ChemostatFcPG-03</b>	<b>Initial Strain</b>	<b>Evolved Strain</b>
Glucose consumption	0.12	0.21
1,2-propanediol production	0.02	0.07
1,2-propanediol and hydroxyacetone production	0.04	0.08

**Table 3S**

<b>ChemostatFcPG-02</b>	<b>Initial Strain</b>	<b>Evolved Strain</b>
Glucose consumption	0.10	0.22
1,2-propanediol production	0.01	0.08
1,2-propanediol and hydroxyacetone production	0.04	0.08

As can be seen, in each of the chemostat preparations, the evolved strain has improved production for the desired product.

**Claims 4, 7-10, 26-29 and 35-39 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 4, 7-10, 26-29 and 35-39 recite the phrase “favours”.**

This rejection is now moot.

The term “favours” has been removed from the claims. Withdrawal is respectfully requested.

**Claims 6 and 27 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 6 and 27 recite the phrase “low sensitivity to inhibition by NADH.”**

The rejection is overcome, at least, for the following reasons.

Those of skill in the art recognize that metabolic efficiency of living organisms, e.g., the enzymatic productivity, is governed to some extent by the redox potential of the cell. As discussed in the specification:

“The present invention makes possible the selection of a pyruvate dehydrogenase complex that is functional in anaerobic conditions and that produces two NADH equivalents by oxidation of glyceraldehyde-3-phosphate to acetate. These NADH equivalents can be re-oxidized only by the pathway of reduction of dihydroxyacetone-phosphate to 1,2-propanediol. The selection of an enzyme complex with low sensitivity to NADH favours a high rate of production of 1,2-propanediol.”

US20070072279 at [0065].

Further, the role of NAD<sup>+</sup>/NADH in maintaining proper redox potential is recognized event to the extent that NAD<sup>+</sup> is considered a coenzyme found in all living cells.

In metabolism, NAD<sup>+</sup> is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is therefore found in two forms in cells: NAD<sup>+</sup> is an oxidizing agent – it accepts electrons from other molecules and becomes reduced, this reaction forms **NADH**, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD<sup>+</sup>.

<http://en.wikipedia.org/wiki/NADH>

Further, the Applicants respectfully submit that the term “sensitivity to inhibition by NADH”. For example, the Office is referred to **Kim Y, Ingram LO, Shanmugam KT.** “Dihydrolipoamide dehydrogenase mutation alters the NADH sensitivity of pyruvate dehydrogenase complex of Escherichia coli K-12”, J Bacteriol.; 2008 Jun;190(11):3851-8. (copy provided herewith for the Examiner’s convenience). Specifically, the paper is concerned with the NADH sensitivity of the pyruvate dehydrogenase complex. The abstract explicitly states “The lower sensitivity of PDH to NADH inhibition.” (Emphasis added). Thus, Applicants respectfully submit that the meaning of the term “low sensitivity to inhibition by NADH” as used in the claim is well understood by those of skill in the art. The rejection being overcome, at least for this reason, withdrawal is respectfully requested.

**Claims 1-14, 16-17 and 22-49 were rejected under 35 U.S.C. § 112, first paragraph, as being containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.**

This rejection has been overcome, at least, for the following reasons.

The claims have been amended herein to recite the use of the invention only with bacterium. Specifically, E. Coli and Corynebacterium. The rejection being therefore overcome, withdrawal is respectfully requested.

#### CLAIM REJECTIONS UNDER 35 U.S.C. § 102

**Claims 1-3, 5-9, 13, 14, 16, 22-24, 26-33, 35-39 and 42-43 were rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 6,303,352 to Cameron et al. (hereinafter “Cameron”)**

This rejection is overcome, at least, for the following reasons.

#### Cameron Does Not Teach The Evolution Of Endogenous Genes

Claim 1 has been rewritten herein to more clearly recite the steps required. Explicit support for the amendment is found in the original claim and throughout the specification at, for example, Example 2, Paragraphs [0203]-[0204], is represented in the figures, especially Figs. 2A and 2B and [0041]-[0050]. Thus no new matter is added by way of these amendments. The instant claims require a four step process. First, an initial strain, which has already been modified by deletion of the *tpiA* gene and at least one gene involved in the conversion of methylglyoxal are deleted. Next, the initial strain is cultured on a growth medium having a simple carbon source for a sufficient time period (comprising many generations) until the culture begins to grow. Since no exogenous genes are transfected into the initial strain, the growth of the culture represent the evolution of genes in the 1,2 propanediol biosynthetic pathway. Then the strain that is responsible for growth of the culture is selected and isolated. In this way, endogenous genes of the bacterium evolve the capacity to provide improved 1,2-propanediol synthase synthase activity.

In contrast, Cameron teaches “a recombinant organisms whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-propanediol.” Col. 2, lines 54-56. First, applicants note that, as used by those of skill in the art the term “recombinant” means:

- 1: relating to or exhibiting genetic **recombination** *<recombinant progeny>*
- 2 a: relating to or containing genetically engineered DNA b: produced by genetic engineering *<recombinant bovine growth hormone>*

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Further, Cameron continues, “More specifically, . . . The recombinant E. coli encode enzymes selected from the group consisting of aldose reductase, glycerol dehydrogenase, or combinations thereof.” Col. 2, lines 56-67. Even more specifically, Cameron states: The invention is also drawn to a synthetic operon which enables the production of 1,2-propanediol in a microorganism transformed to contain the operon. The operon includes one or more genes whose encoded gene products catalyze the reduction of methylglyoxal to 1,2-PD and a promoter sequence operationally linked to the one or more genes. Thus, Applicants, respectfully submit that Cameron teaches away from the present invention.

#### **Cameron Explicitly Teaches Away From The Instant Invention**

Further, the Office cites to col. 6, lines 16-42 of Cameron for the proposition that it teaches the same structure and function of the instant invention, Applicants note first, that it does not teach the elements of the instant claims because it teaches several different mutants that can be used as “host cells” such as the AA200 ΔTP mutant that can be transformed with “exogenous genes”. Col. 6, lines 34-51. Thus, this passage too teaches against the instant invention. While there is no question that Cameron cannot anticipate the instant invention, there can be no question either than Cameron would not make the instant invention obvious in light of the foregoing statements which, when read by one of skill in the art teach that in order to have non-native PD production one would need to have at least the TP mutant transformed with any number of exogenous genes.

In addition, though Cameron does discuss the strain AA200 which is stated to be tpiA deficient, the strain used by Cameron for transformation is generally AG1. However, Applicants

do note that Cameron does use the AA200 strain to produce 1,2-PD but only when transformed with the pSEARX plasmid encoding the gene for aldose reductase. Therefore, Cameron cannot anticipate the instant invention because Cameron does not teach (a) an initial bacterial strain comprising a deletion of the tpiA gene and at least one gene involved in the conversion of propanal into lactate; (b) Cameron does not teach culturing the initial strain for a time period of time until the culture grows; (c) Cameron does not teach evolution of any strain to provide an evolved strain that is improved in 1,2-PD activity; and, (d) lacking an evolved strain, Cameron cannot isolate and select it. Schematically this can be show as:

**Present invention:**

$$M_O \rightarrow M_I \rightarrow M_E$$

$M_O$  represents a wild-type Microbial precursor

$M_I$  represent the initial microorganism having tpiA and one other MG gene deleted; and

$M_E$  represents the evolved microorganism that is not obtained until many generations of  $M_I$  culture.

**Cameron, in contrast teaches**

$$M_O \rightarrow M_I$$

$M_O$  represents a wild-type;

$M_I$  represent the mutant microorganism

Therefore, Cameron, lacking steps, lacking the required deletions from the genome and teaching that exogenous genes are required, can not only, not anticipate the present invention, Cameron does not suggest or make obvious the present invention. Therefore, for these reasons alone the rejection over Cameron is overcome and should be withdrawn. Applicants respectfully request same.

**Comparison With Prior Art Enzymes Are Not Required**

As discussed above, the Cameron discusses, at most use of TP deletion mutants together with the transfection of exogenous genes such as aldose reductase. While Cameron does not specify the source of this exogenous gene its sequence must be known and published if it were used in a transfection vector. Further, the instantly rejected claims are directed to methods of preparing evolved microorganisms. It is therefore unclear what the Office would need

experimental facilities for. However, Applicants do note that if the Office is referring to the mutation that was evolved in the endogenous *lpd* gene recited in claim 17, (which is not named in the instant rejection nor in the following 103 rejection) the sequence was provided to the Office for searching. Applicant notes that the Office does have facilities for such searching. In addition, Applicants also note that such facilities are also available at NCBI Nucleotide database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>). Therefore the rejection is overcome and should be withdrawn.

Further, as discussed above, the Office states that Cameron comprises a deletion of the *tipA* gene (sic) and a deletion of the glyoxalase genes.” As discussed above, this is not a correct statement. Cameron does not teach a combination of the two deletion mutants, Cameron teaches either one mutant or the other transfected with exogenous genes. There is absolutely no discussion in Cameron that a double deletion of endogenous genes would be helpful in attaining production of 1,2-PD. There is absolutely no discussion that 1,2-PD could be produced without the introduction of exogenous genes. Therefore, for this reason alone the rejection is overcome and should be withdrawn. Applicants respectfully request same.

#### CLAIM REJECTIONS UNDER 35 U.S.C. § 103

**Claims 4, 10-12, 25, 34, 40-41 and 46-49 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Cameron as applied to claims 1-3, 5-9, 13-14, 16, 22-24, 26-33, 35-39 and 42-43, and further in view of Altara et al. and Bermejo et al.**

#### Altara And Bermejo Do Not Rectify The Deficiencies Of Cameron

Claims 4, 10-12, 25, 34, 40-41 and 46-49 are dependent on claims 1 and 22, as discussed above, Cameron does not teach the elements of the independent claims and in fact, teaches away from those claims. Therefore, the addition of Altara and Bermejo cannot rectify these deficiencies.

#### Altara Teaches Away From The Present Invention

“We have also explored the development of a complete pathway to 1,2-PD. This is an important approach for several reasons. A cassette expressing the complete pathway to the desired product eliminates the need to rely on the host's native genes and regulations to synthesize the desired enzymatic activities. A complete pathway also allows better control of the expression of each individual gene involved in the pathway and allows more flexibility in

transferring the pathways to other hosts.” Altara at 945, 1<sup>st</sup> Col., 3<sup>rd</sup> Para.) Therefore, Applicants respectfully submit that one of skill in the art would not rely on the host’s native genes as is true in the present case. Further, Altara merely reinforces Cameron to the extent that the solution to the production of 1,2-PD in microorganisms is identified as requiring the introduction of exogenous genes. Therefore, for this reasons alone, the rejection of claims 4, 10-12, 25, 34, 40-41 and 46-49 over Cameron in view of Altara and Bermejo is overcome and should be withdrawn. Applicants respectfully request same.

In addition, applicants note that Altara does not mention *tpiA*. Rather, Altara is concerned with providing a strain that is acceptable to express “a complete pathway to 1,2-PD from the glycolytic intermediate”. Further, Altara discusses the need for the coexpression of multiple genes *gldA*, *mgs*, and *adhI* when compared to the expression of *adhI* alone. (P. 943, 2<sup>nd</sup> Col., 1<sup>st</sup> Para.). Thus, one of skill in the art would not consider the efficacy of merely deleting genes of a microorganism and requiring that microorganisms to evolve so as to grow in the particular culture conditions. Therefore, for this reason alone, because Altara does not remedy the deficiencies of Cameron, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

**Bermejo Does Not Rectify The Deficiencies Of Cameron And Altara**

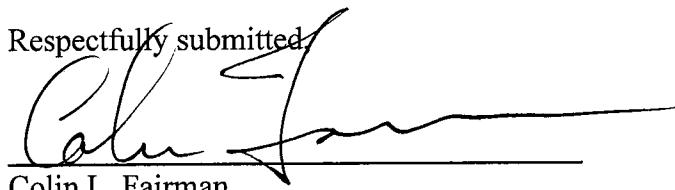
As discussed above, independent claims 1 and 22 require an initial strain of bacteria wherein the microorganism comprises a deletion of the *tpiA* gene and a deletion of at least one gene involved in the conversion of MG into lactate. As discussed above, neither Cameron nor Altara teach the use of such a strain. Further, as discussed above, claim 1 further has multiple steps required. Bermejo is solely concerned with the transfection of E Coli with clostridia genes. To that extent three E. Coli strains are listed in Table 1 of Bermejo. These strains include a wild type and ER2275 and MC1060. None of these strains contain a knockout f the *tpiA* gene and any other gene in the MG to lactate pathway. Therefore, Bermejo does not remedy the defects of Cameron and Altara. The rejection over Cameron in view of Altara and Bermejo is therefore overcome. Withdrawal is respectfully requested.

**CONCLUSION**

In view of the foregoing, it is respectfully submitted that each of the pending claims is in condition for allowance, and a Notice of Allowance is earnestly solicited. The Examiner is invited to contact the undersigned attorney at (612) 321-2237 with any questions, comments or suggestions relating to the referenced patent application.

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Respectfully submitted



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